

CLAIMS

1. A method for controlling the expression of heterologous genes in microorganisms associated with cells of higher organisms consisting of:
 - 5 a. a regulatory system that can be controlled by a chemical effector;
 - b. a host microorganism that contains the regulatory system capable of interacting with or associating with cells of higher organisms;
 - c. an effector chemical compound that is a salicylate or salicylate derivative that can be transported through the associated higher
10 organism and activate or repress the expression of the modified host microorganism.
2. The method claimed in claim 1, wherein at least one of the regulatory proteins is controlled by salicylate, anthranilate, 2-acetyl salicylate, 4-
15 chloro-salicylate, 5-chloro-salicylate, 3,5-dichloro-salicylate, 5-methoxy-salicylate, benzoate, 3-methyl-benzoate, 2-methoxy-benzoate, 3-methyl-salicylate, 4-methyl-salicylate, 5-methyl-salicylate, any other salicylate derivative that conserves the carboxylic C-1 group in the aromatic ring, or mixture of the same.
- 20 3. The method claimed in claim 2, wherein the regulatory system consists of at least one regulatory protein that belongs to the LysR/NahR family of regulators.
4. The method as claimed in claim 2, wherein the regulatory system
25 consists of at least one derivative of the XylS/AraC family of regulators.
5. The method as claimed in claim 2, wherein the regulatory system consists of a regulatory protein belonging to the MarR family of regulators.

6. The method as claimed in claim 2, wherein the regulatory system consists of a system of nahR/Psal expression, or mutants of the same elements.

7. The method as claimed in claim 2, wherein the regulatory system
5 consists of a system of xylS/Pm expression, or mutants of the same elements, capable of responding to any of the chemical compounds cited in claim 2.

8. The method as claimed in claim 2, wherein the regulatory system consists of a genetic cascade circuit that consists of:

- 10 a. the transcriptional regulator NahR, or a mutant form of the same, and the transcriptional regulator XylS, or a mutant form of the same, wherein the transcriptional regulators are placed in hierarchical order in such a way that the transcriptional regulator NahR, or a mutant form of the same, stimulates the expression of transcriptional regulator XylS, or
15 a mutant form of the same, and wherein the transcriptional regulator NahR, or a mutant form of the same, and the transcriptional regulator XylS or a mutant form of the same, respond to the same inducer;
- b. a terminal target promoter, wherein said terminal target promoter is characterized by its dose-dependent sensitivity to the transcriptional
20 regulator XylS, or a mutant form of the same.

9. A method as claimed in claim 1 wherein the cell capable of associating with higher organisms and containing the system regulating heterologous gene expression is a prokaryotic cell.

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10. A method as claimed in claim 9 wherein the prokaryotic cell is a gram-negative bacteria.

11. A method as claimed in claim 10 wherein because the gram-negative
30 bacterial cell is a bacteria of the genus Salmonella.

12. A method as claimed in any of the above claims 9 to 11 that has been developed to control the expression of therapeutic proteins or a diagnostic protein in an attenuated bacteria associated with cells of higher organisms.

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13. A method as claimed in claim 12 wherein the expression of a heterologous antigen in an attenuated bacteria associated with cells of higher organisms is controlled for use as a recombinant live vaccine.

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14. A method as claimed in claim 12 wherein the expression of at least one antitumoral protein that has a certain tropism for tumoral cells in a higher organism is controlled in an attenuated bacteria associated with cells of said higher organism.

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15. The method as claimed in claim 14 wherein the antitumoral protein is selected from a group consisting of an interleukin, cytokine, toxin, cytotoxic protein, or antiangiogenic protein.

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16. The method as claimed in claim 9 wherein the bacteria also contains a regulatory system sensitive to salicylate or its derivatives and a target promoter, which controls the expression of a suicide gene that encodes a product toxic to the host cell.

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17. The method is used as claimed in claim 9 to study the genes implicated in pathogenesis using bacteria with conditional phenotypes.

18. The method is used as claimed in claim 16 for the selective elimination of microorganisms in bioreactors.

19. The method is used as claimed in claim 16 for the selective elimination of cells that can be selectively infected with a bacteria or virus that produces a toxic process or a biomolecule capable of triggering apoptosis or any other mechanism of cell death in malignant cells.

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20. The method used to control the expression of heterologous therapeutic proteins or heterologous diagnostic proteins in microorganisms associated with cells of higher organisms consists of:

- 10 a. a bacterial cell that contains a regulatory system that can be controlled by salicylate or salicylate derivative as a chemical effector, which is associated specifically to cells of a higher organism;
- b. the induction of the expression of said heterologous therapeutic proteins or heterologous diagnostic proteins by administering salicylate or salicylate derivatives to the higher organism hosting the modified cell.

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21. The method as claimed in claim 20 wherein the regulatory system is selected from among the group formed by at least one regulatory protein that belongs to the LysR/NahR family of regulators, at least one derivative of the XylS/AraC family of regulators, at least one regulatory protein that belongs to the family of MarR regulators, at least one nahR/Psal regulatory system, or mutant of the same elements, at least one system of xylS/Pm expression, or mutant of the same elements, a genetic cascade circuit that consists of the transcriptional regulator NahR, or a mutant form of the same, and the transcriptional regulator XylS, or a mutant form of the same, or a combination of the above.

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22. A method as claimed in any of the above claims 20 to 21 wherein said heterologous therapeutic protein is a heterologous antigen, an antitumoral protein, or mixture of the same.

23. The regulatory system of heterologous gene expression in bacteria is associated with tumoral cells of a higher organism and controlled by salicylate or salicylate derivatives to regulate the expression of at least one antitumoral protein.

5 24. A use as claimed in claim 23 wherein the antitumoral protein expressed is selected from the group consisting of an interleukin, cytokine, toxin, cytotoxic protein, or antiangiogenic protein.

10 25. The use of a system regulating heterologous gene expression in bacteria associated with cells of a higher organism that is controlled by salicylate or salicylate derivatives to regulate the expression of a heterologous antigen for use as a recombinant live vaccine.

15 26. The use of a regulatory system of heterologous gene expression in bacteria associated with cells of a higher organism, as claimed in any of claims 23 to 25, wherein said regulatory system is selected from a group consisting of at least one regulatory protein pertaining to the LysR/NahR family of regulators, at least one derivative of the XylS/AraC family of regulators, at least one regulatory protein pertaining to the MarR family of regulators, at least one nahR/PsaI regulatory system, or mutants of the same elements, at least one xylS/Pm regulatory system, or mutants of the same elements, a genetic cascade circuit that consists of the transcriptional regulator NahR, or a mutant form of the same, and the transcriptional regulator XylS, or a mutant form of the same, or a combination of the above.

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27. The modified bacteria that contains the regulatory system used is selected from the group consisting of at least one regulatory protein belonging to the LysR/NahR family of regulators, at least one derivative of the XylS/AraC family of regulators, at least one regulatory protein belonging to the MarR family of regulators, at least one nahR/PsaI regulatory system, or mutants of the same

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elements, at least one system of xylS/Pm expression, or mutants of the same elements, a genetic circuit cascade that consists of the transcriptional regulator NahR, or a mutant form of the same, and the transcriptional regulator XylS, or a mutant form of the same, or a combination of the above, in the method of control
5 of heterologous gene expression in microorganisms associated to cells of higher organisms. as claimed in claim 1.